INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 10, No. 6, p. 246-252, 2017

RESEARCH PAPER OPEN ACCESS

Detection, quantification and decontamination of pesticide residues in dry fish

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Key words: Dry fish, decontamination, pesticide, residue.

http://dx.doi.org/10.12692/ijb/10.6.246-252 Article published on June 30, 2017

Abstract

This study was taken on to detect, quantify and decontamination of the residue of organochlorine pesticides in ten popular dry fish samples collected from different regions of Bangladesh. Out of 60 dry fish, thirty (30) percent dry fish were contaminated with endrin (0.05-0.18ppm), heptachlor epoxide-0.07ppm and endrin ketone-0.09ppm. Among those, fifteen percent samples were in above MRL (above 0.1ppm). Most of the sample of Loitta and Kanchki from Bogra were contaminated. Churi, Chingri, Hangor and Chanda collected from different location contained no detectable organochlorine residue. However, these detected organochlorine residues were considerably unsafe for consumer. Hence, it is required to minimize the left over residue from contaminated dry fish by using a technique which can be adopted easily at home. Therefore, to evaluate certain methods for removal of pesticide residues from endrine contaminated dry fish by using some household product. Estimation of residues was done using Gas Chromatography equipped with Electron Captured Detector. The results indicated that dipping in 2% vinegar solution for 15 minutes followed by washing with tap water was found to be more effective in reducing endrin pesticide (66%) when compared with other treatment solutions (0-54%) in Loitta fish. On the other hand, combination of two treatments (dipping in 2% vinegar+cooking) was reduced pesticide residues upto 98 percent from Loitta fish. This study helped to standardize simple cost effective strategies to eliminate harmful pesticides from dry fish which could be practiced by home makers.

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Introduction

In Bangladesh, fish is about 80% of the daily-required animal protein comes from fish source (Karim, 1987) of which a remarkable part comes from dried fishes. Being riverside country and the existence of the Bay of Bengal at the south of Bangladesh, it has a good natural production of fish. Distribution of these fishes to the consumers or at least to the fish markets is always troublesome due to communication problem. So the fishermen have to process all the excess fish with sun drying, the traditional and low cost procedure (Islam, 1999). This procedure is usually made in the remote coastal isolated islands and in inland depressions where chilling and freezing facilities are lacking. The finally dried fish products are generally stored in a dump warehouse nearby coastal towns. In addition to this, the weather is humid particularly during the monsoon period and the dry fishes absorbed moisture rapidly that the fish becomes suitable for infestation by beetles and mites. Most unexpected causes of infestation are that the fishermen do not dry fishes properly due to loss of weight i.e., the fishermen want more profit selling the dry fishes in weight.

However, to protect the products form the infestation of insects, the processors, whole sellers and retailers often use various harmful insecticides and fungicides indiscriminately. Use of insecticides like DDT, Nogos, and Robral etc. to avoid insect infestation was reported during market surveyin Netrokona, Mohongonj and Mymensingh (Islam, 1999). Under such circumstances they use a mixture of organ chlorine (DDT and heptachlor) insecticides (Bhuiyan *et al.,* 2008). Some analyses in Bangladesh show alarming pollutants in fish like DDT and heptachlor (BCAS, 1990). In Kuakata (a fish processing zone in Bangladesh), high level of DDT powder (locally known as white powder) is used though Bangladesh banned the 'dirty dozen' in 1997 (Barua, 2007).

So, dry fish have revolved into a foremost source of life-taking poisonous substances and their residues into the human body though it is a high protine delicious food. Presence of these persistent chemicals as residue prompts multiple health complexities ranging from mild allergies to deadly diseases.

To lessen dietary exposure to pesticides, it is important to detect and quantify the residue in dry fish as well as discover the strategies that successfully support in reducing the residue content at different level. It has been reported that commercial and household processing such as washing, peeling, cooking, blanching and concentrating can reduce residue levels in food, which further minimize the impact of hazards on human health (Soliman, 2001; Zohair, 2001; Byrne and Pinkerton, 2004; Pugliese *et al.,* 2004; Zhang *et al.,* 2007). The present study was taken on to detect and quantify the amount of leftover residue of insecticide in different dry fish samples collected from different local market of Bangladesh. In addition, it also efforts to determine the detoxification strategy of contaminated dry fish, which is not done before.

Materials and methods

Chemicals and Reagents

Certified Reference Materials (CRM) of organochlorine pesticide mix (ά-BHC, δ- BHC, β-BHC, γ- BHC, Heptachlor, Aldrin, Heptachlor Epoxide, γ- Chlordane, ά- Chlordane, ά- Endosulfan, 4, 4" DDE, Dieldrin, Endrin, 4,4' DDD, β-Endoulfan, 4, 4'DDT, Endrin Aldehyde, Methoxychlor, Endrin Ketone) were used in the present study having purity > 99.99 per cent were obtained from M/s Sigma Aldrich, Germany through SF Scientific, Dhaka and stored in a freezer at low temperature, without light and moisture.

Analytical graded n-hexane, anhydrous sodium sulphate (Na_2SO_4) manufactured by Scharlau were purchased from M/s Sigma Aldrich, Germany through SF Scientific, Dhaka keeping in anhydrous condition in the laboratory. A weighed amount of analytical grade material of each pesticide was dissolved in a minimum quantity of distilled acetone and diluted with n-hexane: toluene (1:1) to obtain a stock solution of 1000mg kg-1.

Dry fish

Ten different types of dry fish samples like Loitta, Kanchki, Mola, Paysha, Chanda, Churi, Chingr, Shidhol, Hangor & Chepa were collected from nine location of Bangladesh like Chittagong, Khagrachori,

Rangpur, Bogra, Dinajpur, Mymensingh, Dhaka, Rajshahi & Khagrachori. Collected dry fishes were analyzed for the quantification of insecticide residues.

Recovery study

The intermediary standards and working standards of 500, 300, 200, 100, 50µg kg-1 were prepared by appropriately diluting the stock solution in acetonitrile and used as standard check in analysis, linearity and recovery studies. The analytical method for estimation of residues of pesticides in dry fish has been validated by conducting recovery studies using control samples. Control samples were fortified with the above mentioned five level of standard.

Decontamination study

Contaminated dried Loitta fishes were divided into eight parts then treated by the following treatments: washing with running water (wrw), dipping in normal water (dnw), dipping in 2% common salt water solution (dsw), dipping in 2% vinegar water solution (dvw), dipping in 1% turmeric powder solution (dtw), dipping in luke warm water (dlw), cooking until soften (cooking), dipping in 2% vinegar solution+cooking (dvw+cooking). Washing-Washing for one minute under running tap water.

Dipping in different solution- Five parts of the samples of dried Loitta fish were dipped in 2% common salt solution (20g common salt dissolved in one litre water), 2% vinegar solution (20ml vinegar diluted in one litre water), 1% turmeric powder solution (10g turmeric powder dissolved in one litre water), Luke warm water (36-40°C) and normal water followed by washing under running water for 1 minute.

Cooking-Dry fishes were dipped in luke warm water + cooking with turmeric, salt and chili etc.

Sample preparation procedure

Extraction and separation

The collected dry fish samples (250g) were cut down into small pices by knife. A sub sample of 20g was taken into a wide mouth jar then 100 ml of hexane was added to it. Anhydrous sodium sulphate (Na_2SO_4) was also added with sample until water was removed from the sample.

The mixture was then macerated with high-speed homogenizer (Ultraturax, IKA T18 basic, Germany) for 2 minutes. The homogenized material were then poured into 250ml conical flask and placed into shaker (Orbital Shaking Incubator, Rexmed, Sweden) for 12hrs continuous shaking. After shaking, the slurry was filtered through a Buchner funnel with suction. The flask and filter cakes were rinsed with 25ml of hexane each. The filtrate was then transferred into 250ml round bottom flask and was dried to 3-5ml by evaporation using a rotary vacuum evaporator (Laborota-4001, Heidolph, Germany). The concentrated filtrate was then transferred into volumetric flask making 10ml in volume. For colour removal, around 20ml methanol was added with 10ml filtrate and shaked vigorously for 3-5 minutes. After shaking, the separatory funnel was set on stand and kept undisturbed for 3-5 minutes. Then the clear part of the solution from the bottom of the separatory funnel was collected in vial which was then centrifuged at 1200rpm for 5minutes (Laboratory Centrifuges, Sigma-3K30, Germany). After centrifuge, supernatant was collected for injection. Before injection, this volume was again cleaned up by High performance liquid chromatography (HPLC) filter (0.2 PTFE) which was ready for injection.

Detection and quantification of insecticide residue in samples

The concentrated extracts were subjected to analysis by GC-2010 (Shimadzu) with Electron Capture Detector (ECD) to detect mentioned organochlorine residue. The capillary column used was AT-1, length 30m, ID 0.25mm and film thickness 0.25µm in case of ECD detector. Nitrogen was used as carrier and make up gas in ECD (table.1).

Prior to the injection of the sample extract, standard solutions of different concentrations of each insecticide were prepared and injected with selected instrument parameters. The samples were calibrated (retention time, peak area etc.) against three to four pointed calibration curve of standard solution of concerned pesticide. Each peak was characterized by its retention time.

Sample results were expressed in ppm automatically by the GC software which represented the concentration of the final volume injected. From this value, the actual amount of pesticide residue present in the sample was determined by using the following formula:

$$\text{Residue in sample (ppm)} = \frac{\text{Conc. obtained in injected volume (ppm) X Quantity of final volume (L)}}{\text{Amount of sample taken (kg)}}$$

Results and discussion

Recovery study

The analytical method was validated in terms of recovery study. The fortification study was carried out by spiking the untreated control samples to determine the recovery levels. The average recoveries of the method for 19 organochlorine pesticides were 80-120%. The recovery %s in this study was equal or higher than 80%, which indicated good and validated analytical procedure. The requirements regarding the process, for the quantitative analysis of pesticide residue are generally considered satisfactory if recovery is over 70% (European commission, 2013).

Determination of Residue

The analytical results of the dry fish samples for the detection of insecticide residue have been summarized in Table 2. Out of sixty dry fish, twenty dry fish were contaminated with Endrin, Heptachlor epoxide and endrin ketone. Among these samples, fifteen percent samples were in above MRL. Most of the residues were contaminated with endrin in dry fish collected from Bogra. Concentrations of endrin were found between range of 0.07-0.12mg/kg, in orderly; 0.11 mg/kg for Loitta, 0.07mg/kg for Kanchki, 0.12mg/kg for Mola, 0.09mg/kg for Paysha and 0.08mg/kg for Chepa respectively in samples collected from Bogra. The lowest concentration of endrin found in paysha at 0.05mg/kg and the highest concentration was found in loitta at 0.18mg/kg collected from Dinajpur and Rangpur respectively. Heptachlor epoxide at 0.07mg/kg and endrin ketone at 0.09mg/kg were detected in chepa and shidhol collected from Rajshahi and Mymensingh accordingly. Loitta, Kanchki, Mola and Chepa collected from Bogra, Chittagong, Rajshahi, Rangpur, Mymensingh, Dhaka and Dinajpur were contaminated with above MRL endrin residue. These obtained results are alarming for Bangladesh. The level of concentration of Organochlorine pesticides like endrin in dry fish is a great unease and also more concern that Persistent Organic Polutants (POPs) pesticides is still now are readily available in spite of official bans or severe restraints. Government may privation the implementation of the legislation fully. People are vending these chemicals may have no idea about restriction. The government of Bangladesh ought to take all the obligatory paces to keep the situation under control. The fisherman should dry dried fish well enough and should package very prudently so that the fish cannot absorb moisture in rainy monsoon, which cause pest attack followed by pesticide use. So, arrangement of awareness program on this area is recommended as farmers could keep preventive measure before using pesticide in dry fish.

Decontamination study

The mean per cent reduction of pesticides of dried loitta fish through different household treatments were showed in Table. 3. From this study, it could be reveal that, washing with running water could reduce up to 35 percent followed by dipping in 2% common salt solution removed 37 percent of endrin residue. On the other hand, dipping in 1% turmeric powder solution and dipping in 2% vinegar could reduce 54 and 66 percent of residue respectively. In addition, most effective treatment was dipping in 2% vinegar plus cooking, which removed up to 98 percent of endrin residue successfully. These results agree with those obtained by Zohair, 2001 in different crop with different pesticide. However, dipping in normal water and Luke warm water reduced only 22 and 0 per cent endrin residue from dried loitta fish. In anticipation of this day no researchers have been study on decontamination of pesticide in the dry fish of Bangladesh. This is much essential to know the decontamination tactics in ingestion level to minimize the residue intake through dry fish.

From this study it could be reveal that, dipping in 2% vinegar plus cooking is the best decontamination strategy for loitta dry fish in removing endrin residue. Although by washing, residues were reduced to some extent but not up to the mark as pesticide residues rapidly spread in to fat and cuticulas of dry fish after spraying.

Thus, washing the dry fish only with water would be insufficient in removing the pesticides. So, available household items could be used easily by homemakers.

Table 1. Instrument parameters for GC-ECD were as follows.

Gas Chromatograph	SHIMADZU-2010
Detector	ECD
Column	RTX-CL Pesticides, 30 meters, 0.25 mm ID
Injector Temperature	250°C
Split Ratio	10.0
Carrier Gas	Nitrogen/Air
Carrier Gas flow	3 ml/min.
Column Oven Temperature	Initial temp. 180°C, @ 5°C /min 220°C, 12 min. hold, 5°C /min 260°C. Total time 28 min.
ECD	330°C
Makeup flow	40 ml/min.

Table 2. Quantity of residue of different pesticides detected in dry fish collected from different location of Bangladesh.

Collected dry fish	Location	Detected residue and level (mg/kg)	MRL (mg/kg)
Loitta	Bogra	Endrin-0.11	0.1
	Chittagong	Endrin-0.10	
	Mymensingh	Endrin-0.09	
	Rajshahi	Endrin-0.13	
	Rangpur	Endrin-0.18	
kanchki	Bogra	Endrin-0.07	
	Chittagong	Endrin-0.06	
	Mymensingh	Endrin-0.12	
	Dinajpur	Endrin-0.09	
	Dhaka	Endrin-0.105	
	Rajshahi	Endrin-0.12	
Mola	Bogra	Endrin-0.12	
	Chittagong	Endrin-0.07	
Paysha	Bogra	Endrin-0.09	
	Dinajpur	Endrin-0.05	
	Mymensingh	Endrin-0.08	
Chepa	Bogra	Endrin-0.08	
	Dinajpur	Endrin-0.13	
	Rajshahi	Heptachlor epoxide-0.07	
Churi	-	-	
Chingri	-	-	
Hangor	-	-	
Chanda	-	-	
Shidhol	Mymensingh	Endrin ketone-0.09	

Table 3. Mean per cent of pesticide reduction from Loitta fish

Treatments	Mean % Reduction of pesticides
Washing with Running Water	35
Dipping in Normal Water	22
Dipping in 2% Common Salt Solution	37
Dipping in 2% Vinegar	66
Dipping in 1% Turmeric powder solution	54
Dipping in Luke warm water	0
Cooking	32
Dipping in 2% Vinegar+Cooking	98

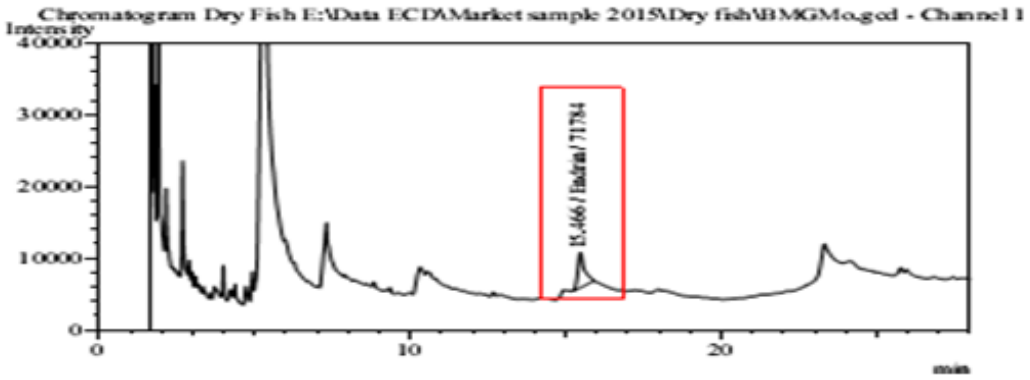

Fig. 1. Chromatogram of pesticide residue in dried mola fish collected from Bogra.

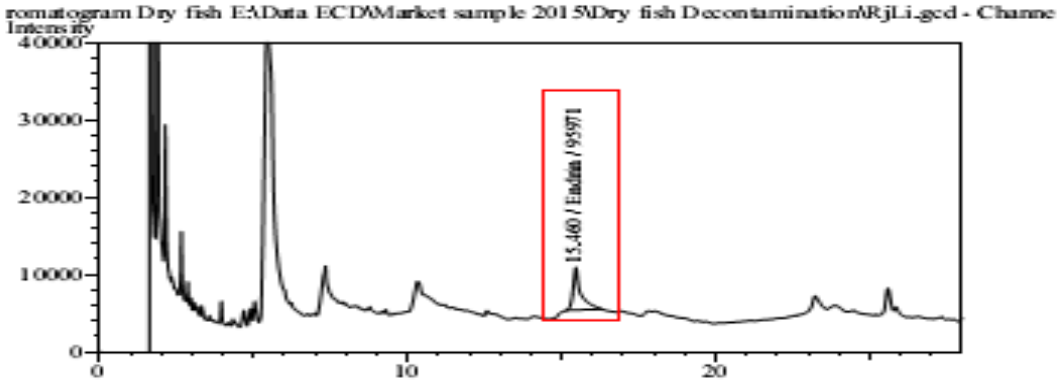

Fig. 2. Chromatogram of pesticide residue in dried loitta fish collected from Rajshahi.

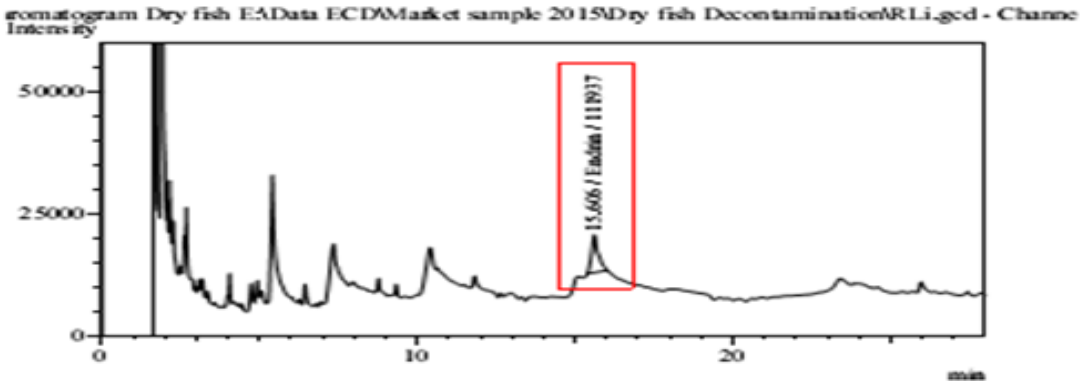

Fig. 3. Chromatogram of pesticide residue in dried loitta fish collected from Rangpur.

Australian Pesticides and Veterinary Medicins Authirity Recommended

*MRL of Endrin : 0.1 ppm

*MRL of Heptachlor epoxide: 0.1 ppm

*MRL of Endrin ketone : 0.1 ppm

*FAO/ IAEA/ WHO recommended

ADI of Endrin : 0.0001 mg/kg body wt

ADI of Heptachlor epoxide: 0.0001 mg/kg body wt. &

ADI of Endrin ketone: 0.0001 mg/kg body wt.

Conclusion

To my best knowledge, this is the first time results revealed on decontamination strategies in dry fish. This primary research will help to homogenize simple cost effective approaches by using household items to eradicate unsafe residues of pesticides from dry fish which could be adept by home makers as well as will inspire other researchers to do further study on it. On the other hand, efforts should be given on restraining the usages of hazardous pesticides for evading any health risk condition and warranting food safety.

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